

## Mechanistic Basis for the Binding of RGD- and AGDV-Peptides to the Platelet Integrin $\alpha\text{IIb}\beta 3$

Kononova O., Litvinov R., Blokhin D., Klochkov V., Weisel J., Bennett J., Barsegov V.  
*Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia*

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### Abstract

© 2017 American Chemical Society. Binding of soluble fibrinogen to the activated conformation of the integrin  $\alpha\text{IIb}\beta 3$  is required for platelet aggregation and is mediated exclusively by the C-terminal AGDV-containing dodecapeptide ( $\gamma\text{C}$ -12) sequence of the fibrinogen  $\gamma$  chain. However, peptides containing the Arg-Gly-Asp (RGD) sequences located in two places in the fibrinogen  $\text{A}\alpha$  chain inhibit soluble fibrinogen binding to  $\alpha\text{IIb}\beta 3$  and make substantial contributions to  $\alpha\text{IIb}\beta 3$  binding when fibrinogen is immobilized and when it is converted to fibrin. Here, we employed optical trap-based nanomechanical measurements and computational molecular modeling to determine the kinetics, energetics, and structural details of cyclic RGDFK (cRGDFK) and  $\gamma\text{C}$ -12 binding to  $\alpha\text{IIb}\beta 3$ . Docking analysis revealed that NMR-determined solution structures of cRGDFK and  $\gamma\text{C}$ -12 bind to both the open and closed  $\alpha\text{IIb}\beta 3$  conformers at the interface between the  $\alpha\text{IIb}$   $\beta$ -propeller domain and the  $\beta 3$   $\beta\text{I}$  domain. The nanomechanical measurements revealed that cRGDFK binds to  $\alpha\text{IIb}\beta 3$  at least as tightly as  $\gamma\text{C}$ -12. A subsequent analysis of molecular force profiles and the number of peptide– $\alpha\text{IIb}\beta 3$  binding contacts revealed that both peptides form stable bimolecular complexes with  $\alpha\text{IIb}\beta 3$  that dissociate in the 60–120 pN range. The Gibbs free energy profiles of the  $\alpha\text{IIb}\beta 3$ -peptide complexes revealed that the overall stability of the  $\alpha\text{IIb}\beta 3$ -cRGDFK complex was comparable with that of the  $\alpha\text{IIb}\beta 3$ - $\gamma\text{C}$ -12 complex. Thus, these results provide a mechanistic explanation for previous observations that RGD- and AGDV-containing peptides are both potent inhibitors of the  $\alpha\text{IIb}\beta 3$ -fibrinogen interactions and are consistent with the observation that RGD motifs, in addition to AGDV, support interaction of  $\alpha\text{IIb}\beta 3$  with immobilized fibrinogen and fibrin.

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